

P:OPERIMRO\ECODYSONE.CIP - 1/7/99

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WE CLAIM:

1. An isolated nucleic acid molecule comprising a nucleotide sequence which encodes or is complementary to a sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide or a bioactive derivative or analogue thereof, wherein said polypeptide is selected from the groups consisting of (i) an EcR polypeptide of a steroid receptor; (ii) the partner protein (USP polypeptide) of a steroid receptor; and (iii) the USP polypeptide of a juvenile hormone receptor; and wherein said polypeptide comprises an amino acid sequence that is at least 40% identical to an amino acid sequence selected from the group consisting of:
 - (a) an amino acid sequence set forth in any one of <400>2, <400>4, <400>6, <400>10, <400>12 or <400>14; and
 - (b) an amino acid sequence encoded by a cDNA present in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568.
2. The isolated nucleic acid molecule according to claim 1, wherein the steroid receptor is an ecdysteroid receptor.
3. The isolated nucleic acid molecule according to claim 2, wherein the ecdysteroid receptor is an insect ecdysone receptor.
4. The isolated nucleic acid molecule according to claim 3, wherein the insect ecdysone receptor comprises the EcR polypeptide of an insect ecdysone receptor or the partner protein (USP polypeptide) of an insect ecdysone receptor.
5. The isolated nucleic acid molecule according to claim 4, wherein the insect is selected from the list comprising dipteran, hemipteran, coleopteran, lepidopteran, and neuropteran insects and ants.
6. The isolated nucleic acid molecule according to claim 5, wherein the hemipteran insect is *Myzus persicae* or a close relative thereof.

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7. The isolated nucleic acid molecule according to claim 6, wherein the insect steroid receptor polypeptide comprises an EcR polypeptide of the *M. persicae* ecdysone receptor having the amino acid sequence set forth in <400>6 or <400>10 or encoded by the cDNA present in plasmid pMpEcR (AGAL Accession No. NM99/04567) or a bioactive analogue or derivative thereof.
8. The isolated nucleic acid molecule according to claim 6, wherein the insect steroid receptor polypeptide comprises an EcR partner protein (USP polypeptide) of the *M. persicae* ecdysone receptor or a USP polypeptide of the *M. persicae* juvenile hormone receptor having or including the amino acid sequence set forth in <400>12 or encoded by the cDNA present in plasmid pMpUSP (AGAL Accession No. NM99/04568) or a bioactive analogue or derivative thereof.
9. The isolated nucleic acid molecule according to claim 5, wherein the dipteran insect is *L. cuprina* or a close relative thereof.
10. The isolated nucleic acid molecule according to claim 9, wherein the insect steroid receptor polypeptide comprises an EcR polypeptide of the *L. cuprina* ecdysone receptor having the amino acid sequence set forth in <400>2 or encoded by the cDNA present in plasmid pLcEcR (AGAL Accession No. NM99/04566) or a bioactive analogue or derivative thereof.
11. The isolated nucleic acid molecule according to claim 9, wherein the insect steroid receptor polypeptide comprises an EcR partner protein (USP polypeptide) of the *L. cuprina* ecdysone receptor or a USP polypeptide of the *L. cuprina* juvenile hormone receptor having the amino acid sequence set forth in <400>4 or <400>14 or encoded by the cDNA present in plasmid pLcUSP (AGAL Accession No. NM99/04565) or a bioactive analogue or derivative thereof.
12. The isolated nucleic acid molecule according to claim 1, wherein the bioactive derivative or analogue comprises a fragment of an EcR polypeptide of an insect ecdysone receptor or a fragment of an EcR partner protein (USP polypeptide) of an insect ecdysone

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receptor, wherein said fragment includes at least one ligand-binding region of said EcR polypeptide or said EcR partner protein (USP polypeptide).

13. The isolated nucleic acid molecule according to claim 12, wherein the ligand-binding region comprises a linker domain of the EcR polypeptide or a linker domain of the EcR partner protein (USP polypeptide).

14. The isolated nucleic acid molecule according to claim 12, wherein the ligand-binding region comprises a hormone-binding domain of the EcR polypeptide or a hormone-binding domain of the EcR partner protein (USP polypeptide).

15. The isolated nucleic acid molecule according to claim 12, wherein the ligand-binding region comprises a linker domain and hormone-binding domain of the EcR polypeptide or a linker domain and hormone-binding domain of the EcR partner protein (USP polypeptide).

16. The isolated nucleic acid molecule according to claim 1, comprising a protein-encoding nucleotide sequence which is at least 40% identical to any one of the nucleotide sequences set forth in <400>1, <400>3, <400>5, <400>9, <400>11 or <400>13 or a complementary nucleotide sequence thereto or the cDNA present in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568.

17. An isolated nucleic acid molecule comprising a nucleotide sequence which encodes or is complementary to a sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide or a bioactive derivative or analogue thereof, wherein said nucleotide sequence is selected from the list comprising:

- (i) a nucleotide sequence having at least 40% identity to any one of the nucleotide sequences set forth in <400>1, <400>3, <400>5, <400>9, <400>11 or <400>13 or a complementary nucleotide sequence thereto;
- (ii) a nucleotide sequence that is capable of hybridising under at least low stringency conditions to any one of the nucleotide sequences set forth in <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11 or <400>13 or to a

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complementary nucleotide sequence thereto;

(iii) a nucleotide sequence having at least 40% identity to a nucleotide sequence of a cDNA present in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568;

(iv) a nucleotide sequence that is capable of hybridising under at least low stringency conditions to a cDNA present in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568; and

(v) a nucleotide sequence that is amplifiable by PCR using a nucleic acid primer sequence set forth in any one of <400>15, <400>16, <400>17, <400>18, <400>19 or <400>20.

18. An isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide and comprises the nucleotide sequence set forth in <400>1 or a complementary nucleotide sequence thereto or the nucleotide sequence of the cDNA present in plasmid pLcEcR (AGAL Accession No. NM99/04566).

19. An isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide and comprises the nucleotide sequence set forth in <400>3 or <400>13 or a complementary nucleotide sequence thereto or the nucleotide sequence of the cDNA present in plasmid pLcUSP (AGAL Accession No. NM99/04565).

20. An isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide and comprises the nucleotide sequence set forth in <400>5 or <400>7 or <400>8 or <400>9 or a complementary nucleotide sequence thereto or the nucleotide sequence of the cDNA present in plasmid pMpEcR (AGAL Accession No. NM99/04567).

21. An isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide or a juvenile hormone receptor polypeptide and comprises the nucleotide sequence set forth in <400>11 or a complementary nucleotide sequence thereto or the nucleotide sequence of the cDNA present in plasmid pMpUSP (AGAL Accession No. NM99/04568).

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22. A method of identifying an isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide comprising:

- (i) hybridising genomic DNA, mRNA or cDNA with a hybridisation-effective amount of one or more probes selected from the list comprising:
 - (a) probes comprising at least 10 contiguous nucleotides in length derived from any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, <400>13, <400>15, <400>16, <400>17, <400>18, <400>19 or <400>20 or a complementary nucleotide sequence thereto;
 - (b) probes comprising at least 10 contiguous nucleotides in length derived from a cDNA contained in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568; and
 - (c) hybridisation probes comprising the nucleotide sequences set forth in any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, or <400>13 or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof which is at least 40% identical to said sequence or complement; and
- (ii) detecting the hybridisation.

23. The method of claim 22 wherein the step of detecting the hybridisation comprises detecting a reporter molecule that is covalently bound to the probe.

24. A method of identifying an isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide comprising:

- (i) annealing to genomic DNA, mRNA or cDNA, one or more PCR primers comprising at least 10 contiguous nucleotides in length derived from the group consisting of:
 - (a) a primer derived from any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, <400>13, <400>15, <400>16, <400>17, <400>18, <400>19 or <400>20 or a complementary nucleotide sequence thereto; and

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(b) a primer derived from a cDNA contained in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568; and

(ii) amplifying a nucleotide sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide in a polymerase chain reaction.

25. A method of identifying an isolated nucleic acid molecule which encodes an insect steroid receptor polypeptide which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide comprising:

(i) amplifying a nucleotide sequence which encodes a steroid receptor polypeptide or a juvenile hormone receptor polypeptide in a polymerase chain reaction using one or more PCR primers comprising at least 10 contiguous nucleotides in length from the group consisting of:

(a) a primer derived from any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, <400>13, <400>15, <400>16, <400>17, <400>18, <400>19 or <400>20 or a complementary nucleotide sequence thereto; and

(b) a primer derived from a cDNA contained in any one of the plasmids deposited under AGAL Accession Nos NM99/04565, NM99/04566, NM99/04567, or NM99/04568;

(ii) hybridising the amplified nucleotide sequence to genomic DNA, mRNA or cDNA with a hybridisation-effective amount of one or more probes selected from the group consisting of:

(a) a probe comprising at least 10 contiguous nucleotides in length derived from any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, <400>13, <400>15, <400>16, <400>17, <400>18, <400>19 or <400>20 or a complementary nucleotide sequence thereto;

(b) a probe comprising at least 10 contiguous nucleotides in length derived from a cDNA contained in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568; and

(c) hybridisation probes comprising the nucleotide sequences set forth in any one of <400>1, <400>3, <400>5, <400>7, <400>8, <400>9, <400>11, or

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<400>13 or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof which is at least 40% identical to said sequence or complement; and

(iii) detecting the hybridisation.

26. The method of claim 25 wherein the step of detecting the hybridisation comprises detecting a reporter molecule that is covalently bound to the probe.

27. The method according to claim 22, further comprising the step of isolating the identified nucleic acid molecule.

28. A genetic construct comprising the isolated nucleic acid molecule according to claim 1 operably linked to a promoter sequence.

29. The genetic construct according to claim 28, wherein the promoter is the SV40, MMTV, polyhedron or p10 promoter.

30. A recombinant or isolated polypeptide comprising a steroid receptor polypeptide or juvenile hormone receptor polypeptide or a bioactive derivative or analogue thereof, wherein said polypeptide:

(i) is selected from the list comprising EcR polypeptide of a steroid receptor, the partner protein (USP polypeptide) of a steroid receptor and the USP polypeptide of a juvenile hormone receptor; and

(ii) comprises an amino acid sequence that is at least 40% identical to any one of the amino acid sequences selected from the group consisting of:

(a) an amino acid sequence set forth in <400>2, <400>4, <400>6, <400>10, <400>12 or <400>14; and

(b) an amino acid sequence encoded by a cDNA present in any one of the plasmids deposited under AGAL Accession Nos. NM99/04565, NM99/04566, NM99/04567, or NM99/04568;

wherein said polypeptide is substantially free of naturally-associated cellular components.

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31. A cell comprising the nucleic acid molecule according to claim 1.
32. A cell comprising the genetic construct according to claim 28.
33. A cell which expresses the isolated or recombinant polypeptide according to claim 30.
34. A method of identifying a modulator of steroid receptor-mediated gene expression or juvenile hormone receptor-mediated gene expression comprising:
 - (i) assaying the expression of a reporter gene in the presence of the recombinant or isolated polypeptide according to claim 30 and a potential modulator; and
 - (ii) assaying the expression of the reporter gene in the presence of the recombinant or isolated polypeptide according to claim 30 and without said potential modulator; and
 - (iii) comparing expression of the reporter gene in the presence of the potential modulator to the expression of a reporter gene in the absence of the potential modulator,wherein said reporter gene is placed operably under the control of a steroid response element (SRE) to which said steroid receptor binds or a promoter sequence comprising said SRE.
35. A method of identifying a potential insecticidal compound comprising:
 - (i) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to claim 30 to a steroid response element (SRE) to which said polypeptide binds, in the presence of a candidate compound; and
 - (ii) assaying the binding directly or indirectly of the recombinant or isolated polypeptide according to claim 30 to a steroid response element (SRE) to which said polypeptide binds, in the absence of said candidate compound; and
 - (iii) comparing the binding assayed at (i) and (ii), wherein a difference in the level of binding indicates that the candidate compound possesses potential insecticidal activity.
36. A method of identifying a candidate insecticidally-active agent comprising the steps of:

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a) expressing an EcR polypeptide of an insect steroid receptor or a fragment thereof which includes the ligand-binding region, optionally in association with an EcR partner protein (USP polypeptide) of an insect steroid receptor or ligand binding domain thereof, optionally in association with an insect steroid or analogue thereof so as to form a complex;

b) purifying or precipitating the complex;

c) determining the three-dimensional structure of the ligand binding domain of the complex; and

d) identifying compounds which bind to or associate with the three-dimensional structure of the ligand binding domain, wherein said compounds represent candidate insecticidally-active agents.

37. A method of identifying a candidate insecticidally-active agent comprising the steps of:

a) expressing a USP polypeptide of a juvenile hormone receptor or a fragment thereof which includes the ligand-binding region, optionally in association with an EcR polypeptide of an insect steroid receptor or ligand binding domain thereof, and optionally in association with an insect steroid or analogue thereof, so as to form a complex;

b) purifying or precipitating the complex;

c) determining the three-dimensional structure of the ligand binding domain of the complex; and

d) identifying compounds which bind to or associate with the three-dimensional structure of the ligand binding domain, wherein said compounds represent candidate insecticidally-active agents.

38. A synthetic compound which interacts with the three dimensional structure of a polypeptide or protein selected from the list comprising:

(i) an EcR polypeptide of a steroid receptor or a fragment or bioactive derivative thereof;

(ii) an EcR partner protein (USP polypeptide) of a steroid receptor or a fragment or bioactive derivative thereof;

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(iii) a USP polypeptide of a juvenile hormone receptor or a fragment or bioactive derivative thereof; and

(iv) a functional receptor or protein complex formed by association of (i) and (ii), wherein said compound is capable of binding to said polypeptide or protein to agonise or antagonise the binding activity or bioactivity thereof.

39. A method of identifying a synthetic compound for insecticidal activity comprising contacting the recombinant or isolated polypeptide according to claim 30 with said compound for a time and under conditions sufficient for binding to occur and detecting said binding using a detection means, wherein the occurrence of binding is indicative of potential insecticidal activity of the compound.

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